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Uranium Bioreduction and Biomineralization

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Abstract

Following the development of nuclear science and technology, uranium contamination has been an ever increasing concern worldwide because of its potential for migration from the waste repositories and long-term contaminated environments. Physical and chemical techniques for uranium pollution are expensive and challenging. An alternative to these technologies is microbially mediated uranium bioremediation in contaminated water and soil environments due to its reduced cost and environmental friendliness (Phillips et al., 1995; Turick et al., 2008). To date, four basic mechanisms of uranium bioremediation - uranium bioreduction, biosorption, biomineralization and bioaccumulation - have been established, of which uranium bioreduction and biomineralization have been studied extensively. The objective of this review is to provide an understanding of recent developments in these two fields in relation to relevant microorganisms, mechanisms, influential factors, and obstacles.

Key words: uranium, bioreduction, bioremediation, biomineralization

1. INTRODUCTION

Uranium is a very common radioactive element and exists in all types of rocks, in varying low concentrations. It is widely distributed in the earth's crust, rocks, and soils at a level of about 2-4ppm (Appleton and Appleton, 2007). Uranium, the heaviest element in nature, exists in three isotopes, uranium 234, uranium 235 and uranium 238, all of which are radioactive. The most abundant isotope is uranium 238 (99.27%) with uranium 235 (0.72%) being the second most abundant. U(IV) and U(VI) are the most important oxidation states in natural environments (Meinrath et al., 2003).

The toxicity of uranium is determined by its chemical and radioactive properties. In general, the more soluble the uranium compound is, the more toxic it becomes. The main radiation risk to humans occurs when uranium compounds are inhaled or ingested. Less soluble uranium compounds are of low to moderate toxicity, while soluble compounds are highly toxic. In general, hexavalent uranium, which forms soluble compounds, is more toxic than less soluble tetravalent uranium minerals (Craig, 2001). All uranium mixtures are considered to be toxic and may cause nephrotoxic effects (Duraković, 1999). The existence of higher levels of uranium in the human body affects renal function and very high concentrations may lead to kidney failure. The main mechanisms of uranium entry into the human body are through ingestion of contaminated water and inhalation of contaminated dust, especially in locations where soil and groundwater are contaminated by radioactive waste (Choy et al., 2006).

Elevated concentrations of uranium are present in uranium mining and milling sites. The major portion of these contaminants will accumulate in either the upper layers of soil or in aquatic sediments (Igwe et al., 2005). Other sources of environmental uranium include nuclear testing in the 1950s and 1960s, and accidental releases, e.g. from Chernobyl in 1986. Such incidents have caused an enormous amount of damage to the environment because of improper radioactive waste disposal, waste dumping and other release incidents (Lloyd and Lovley, 2001). Water seepage is a persistent environmental issue at most abandoned mine sites, and this continues to influence the quality of the natural environment, affecting surface and ground waters, and posing a health risk to humans (Hill, 2004).

In soil and groundwater environments, aerobic and anaerobic microorganisms are ubiquitous. They may interact with uranium via different mechanisms such as bioreduction, biomineralization, biosorption and bioaccumulation, among which uranium bioreduction has been studied extensively and successfully demonstrated in contaminated field sites (Anderson et al. 2003; Gihring et al. 2011; Williams et al. 2011). Due to its stability in varied environmental conditions, uranium biomineralization, which may also result from bioreduction, has also been the subject of much research. In addition to bacteria, fungi are also capable of uranium biotransformations (Fomina et al., 2007, 2008; Liang et al., 2015, 2016). The aim of this review is to outline some recent developments in uranium bioreduction and biomineralization research with bacteria and fungi regarding mechanisms, influential factors, and obstacles to application.

2. URANIUM SPECIES AND MOBILITY

In natural environments, uranium is present as various chemical species such as oxides, precipitates, complexes and natural minerals, as well as existing in the elemental form. Hexavalent uranium U(VI) and tetravalent uranium U(IV) are the most common oxidized forms of uranium in natural systems (Rai et al., 2003). Uranium is present as uraninite, (formerly pitchblende), and can coprecipitate with carbonate, phosphate, silicate and vanadate in ores (Francis et al., 1994; Harper and Kantar, 2008). Uraninite is the most common uranium oxide and may exist as UO_2 (U(IV)) or triuranium octaoxide (U_3O_8) forms; the latter contains a mixture of U(IV) and U(VI). U(VI) occurs in schoepite and other minerals in oxidized environments (Kashparov et al., 1999). Meta-autunite, phosphuranylite, and uranyl hydroxide (schoepite) are the primary U(VI) precipitates in uranium-contaminated soils and sediments at the U.S.DOE Fernald site (Morris et al., 1996).

The mobility of uranium species in the environment depends on its speciation and geochemical factors including pH, redox potential, hydrolysis, dissolution, complexation and sorption. Under usual oxidizing conditions, uranium typically exists in aqueous U(VI) forms, predominantly as the free uranyl ion (UO_2^{2+}) in acidic conditions (pH<5), and hydroxyl complexes such as $UO_2(OH)^+$, $UO_2(OH)_2$ and $UO_2(OH)_3^-$ in slightly acidic environments (pH 5.0-6.5) and in the absence of complexing ligands such as dissolved carbonate, sulfate, and phosphate (Langmuir, 1997; Haas et al., 1998). Under low pH conditions, aqueous U(VI) can strongly adsorb to manganese oxides and ferric oxides (Waite et al., 1994b; Han et al., 2007). At pH 5.5 and 7.0, about 80% and 98% of U(VI) was adsorbed to ferric oxide-rich soil in a uranium contaminated site at Oak Ridge, TN, USA (Barnett et al., 2002). In higher pH environments (pH≥7), and in the presence of elevated concentrations of carbonate, UO_2^{2+} forms strong aqueous complexes, such as UO_2CO_3, $(UO_2)_2CO_3\ (OH)^{3-}$, $UO_2(CO_3)_2^{2-}$, and $UO_2(CO_3)_2^{4-}$, thereby, greatly enhancing U(VI) solubility in carbonate-containing aquatic environments (Langmuir, 1997; Ulrich et al., 2009). These complexes are neutral or anionic, and are poorly adsorbed to mineral surfaces such as Fe(III) and Al(III) oxyhydroxides (Waite et al., 1994b; Katsoyiannis, 2007). Through formation of anionic uranyl tricarbonate [$UO_2(CO_3)_3^{2-}$], the mobility of uranyl increases above pH 8 (Waite et al., 1994a). In addition, the presence of calcium may promote the formation of calcium–uranyl–carbonate complexes, further inhibiting U(VI) sorption (Stewart et al., 2010). The formation of highly stable aqueous Ca–uranyl–carbonate species is likely to decrease the extent of U(VI) reduction since uranyl hydroxyl species are more easily reduced than uranyl carbonates (Brooks et al., 2003; Fox et al., 2013). Other than carbonates, other ligands such as sulfate, phosphate, nitrate, and chloride also complex with U(VI) in acidic environments. The complexation affinity of U(VI) with these inorganic ligands decreases in the order $CO_3^{2-}>PO_4^{3-}>SO_4^{2-}>Cl^->NO_3^-$ (Harper and Kantar, 2008). Furthermore, U(VI) also forms soluble complexes with organic ligands such as citrate and oxalate (Hefnawy et al., 2002). Dissolved U(VI) is considerably more bioavailable for reduction than adsorbed and precipitated or solid-phase U(VI) (Liu et al., 2006). In short, the mobility of U(VI) in aquatic environments is likely to be driven mainly by pH, U(VI) speciation, ligand types, and their complexation reactions.

3. URANIUM BIOREDUCTION

3.1 Reductive microorganisms

Many species of prokaryotes can reduce U(VI) to U(IV) (Suzuki and Suko, 2006). The most common Fe(III)-reducing bacteria are able to use U(VI) as an alternative electron acceptor and reduce it to insoluble U(IV) minerals. In addition, sulfate-reducing bacteria can reduce U(VI) to U(IV) (Lovley et al., 1991; Lovley et al., 2004). Fe(III) reducers are related to uranium reducing *Geobacter uraniireducens* and *Geobacter daltonii*, while sulfate reducers are identified as members of e.g. *Desulfovibrio*, *Desulfobacterium* and *Desulfotomaculum* genera (Akob et al., 2012). In general, *Geobacter* species dominate the anaerobic microbial community during U(VI) bioreduction (Yunjuan Chang et al., 2005; Chandler et al., 2010), and are found in abundance with various electron donors including acetate, lactate, and glucose (Snoeyenboswest et al., 2000). Initial bioreduction of uranium was related to enrichment of *Geobacter* species with sulfate reducers becoming predominant after 30-50 days incubation (Anderson et al., 2003; Barlett et al., 2012b). However, there was little competition between *Geobacter* spp. and sulfate reducers when sufficient acetate was present (Barlett et al., 2012b).

In natural environments, it is often difficult to determine which microbes are responsible for U(VI) reduction due to the presence of other electron acceptors such as nitrate, and various metal ions, which also support anaerobic respiration. However, it is common to ascribe a role in U(VI) reduction to the most dominant microbes in the environment (Williams et al., 2011). Many environmental factors (e.g., pH, salinity, temperature, redox potential, organic substrates, contaminants) influence which microorganisms predominate during in-situ U(VI) bioremediation (Vishnivetskaya et al., 2010; Barlett et al., 2012b). *Desulfovibrio* spp. are dominant in a sulfate-reducing enrichment, while *Clostridium* spp., *Ferribacterium* spp., and *Geothrix* spp. are dominant in an iron-reducing enrichment (Cardenas et al., 2008; Boonchayaanant et al., 2009). *Geobacter*, *Desulfuromonales*, *Desulfovibrio*, *Desulfosporosinus*, *Anaeromyxobacter*, and *Acidovorax* spp. were enriched with ethanol and acetate (Luo et al., 2007; Cardenas et al., 2008), while *Geobacter* spp. and acetogens such as *Clostridium* and *Desulfosporosinus* spp. were enriched by ethanol and methanol (Madden et al., 2009). *Comamonadaceae*, *Geobacteriaceae*, and *Desulfobacterales* were associated with U(VI) reduction when enriched with emulsified vegetable oil (EVO) (Gihring et al., 2011). *Geobacter* species were the predominant "*Geobacteraceae*" in subsurface groundwater, whereas *Desulfuromonas* species predominated in saline groundwater (Finneran et al., 2002). *Ralstonia* and *Dechloromonas* spp. were widely found at low nitrate neutral pH sites, while *Castellaniella* and *Burkholderia* spp. were present at acidic high nitrate uranium contaminated sites (Spain and Krumholz, 2011). *Pseudomonas* sp., *Pantoea* sp., and *Enterobacter* sp. are able to reduce U(VI) under pH 5-6 (Chabalala and Chirwa, 2010). Among the sulfate reducers, some of them have been reported to reduce U(VI), while some of them do not. For example, *Desulfovibrio and Clostrodium* sp. can effectively reduce U(VI) (Tapia-Rodriguez et al., 2010), while *Desulfobacter* and *Desulfotomaculum* sp. enriched with acetate did not reduce U(VI) (Lovley et al., 1993a). Some microorganisms related to U(VI) bioreduction are listed in Table 1.

Fungi are also important components of subsurface microbial populations and may tolerate higher uranium concentrations than many bacteria (Mumtaz et al., 2013). However, it is speculative that any fungi may be able to reduce U(VI) to U(IV) in the absence of any relevant research to date (Gadd and Fomina, 2011).

3.2 Reductive mechanisms

The pathway of electron flow from electron donors to U(VI), the number of electrons transferred to U(VI), the enzymes and genes involved in U(VI) reduction, and the competition between U(VI) and other electron acceptors have been key areas for research into processes of U(VI) reduction. Bacterial pili have been proposed to transfer electrons from the cell to electron acceptors due to their high conductivity (Reguera et al., 2005). Further, the participation of these nanowires in U(VI) reduction is implied from the location of U(IV) precipitates on cells and the formation of "needle-like structures" with precipitated uraninite on cell surfaces of *D. desulfuricans* G20 (Marsili et al., 2005). U(VI) reduction to U(IV) seems to require two electrons. However, Renshaw et al. (2005) suggested a single-electon reduction of U(VI) to U(V), which could form U(IV) and U(VI) by disproportionation. Many researchers have confirmed uraninite located both on the outer membrane and concentrated in the periplasm of bacterial cells (Liu et al., 2002a, b; Lloyd et al., 2003). The *dsr* and *mcr* genes increase under sulfate-reducing conditions while c-type cytochrome genes are primarily associated with *Geobacter* sp. (Liang et al., 2012). U(VI) was preferentially reduced to U(IV) by *G. metallireducens*, with abiotic transfer of electrons to F(III) oxide (Nevin and Lovley, 2000). Introduction of U(VI) into the reaction system decreased F(III) reduction, implying that uranium was the preferred terminal electron acceptor compared to Fe(III) (Neiss et al., 2007). However, the results of Bruce et al. (2000) contrast with the above reports in that Fe(III)-reducing microorganisms preferentially reduced Fe(III) over U(VI). When Fe(III) was depleted, sulfate reducers preferentially reduced U(VI) prior to reducing sulfate because U(VI) reduction is more energetically favourable than sulfate reduction (Coleman et al., 1993; Lovley et al., 1993a). It is still questionable whether electron acceptors such as U(VI), Fe(III), nitrate and sulfate are reduced following the order of their redox potential. If not, other factors that decide electron transfer priority among such ions should be clarified. A schematic illustration of U(VI) reduction is shown in Figure 1.

3.3 Influential factors

Many factors including oxidants, electron donors, U(IV) end-products, pH, redox potential and others may affect U(VI) reduction efficiency and bioreduced U(IV) stability.

3.3.1 Oxidants

In natural environments, oxidants such as oxygen, nitrate, sulfate, Fe(III) and Mn(III,IV) may compete for electrons with U(VI), affecting U(VI) reduction efficiency and bioreduced U(IV) stability.

Both oxygen states (dissolved or gaseous) and concentration may affect bioreduced U(IV) stability. Nearly all bioreduced U(IV) was reoxidized within 120 days when media was gassed with oxygen (Komlos et al., 2008), while another study showed that 88% of bioreduced U(IV) was remobilized after exposure to dissolved oxygen within 54 days (Moon et al., 2007). In contrast, only 17% of bioreduced U(IV) minerals were remobilized in one month after exposure to 1-2 mg/l of dissolved oxygen in groundwater sediments from the Rifle site (N'Guessan et al., 2010). Only 7% of bioreduced U(IV) in sediment samples from the Hanford site was remobilized after exposure to oxic river water for 50 days. The remaining 93% of bioreduced uranium was found to be as nanoparticulate uraninite which is resistant to reoxidation at low dissolved oxygen concentrations (Ahmed et al., 2012). It is interesting to note that addition of oxygen to a *Desulfovibrio*-dominated sulfate-reducing process led to an almost complete reoxidation of bioreduced U(IV). However, this had a limited effect on the *Clostridium*-dominated iron-reducing process (Luo et al., 2007).

Nitrates have a higher electron potential and are more energetically favourable than U(VI) and F(III). Therefore, the presence of nitrate inhibits the development of conditions for U(VI) reduction (Istok et al., 2004). In addition, a high concentration of nitrate can inhibit the growth and metabolism of SRB and affect the treatment efficiency of uranium wastewater (Hu et al., 2011). U(VI) reduction by *D. desulfuricans* was slightly inhibited by the presence of 190 mM nitrate, but not by nitrate concentrations lower than 95 mM (Ganesh et al., 1997). The presence of nitrate is preferential at low pH conditions because consequent denitrification produces OH^- and HCO_3^- thereby neutralizing the pH and stimulating metal reduction (Law et al., 2010; Thorpe et al., 2012). After U(VI) reduction, total reoxidation of U(IV) was achieved when *Pseudomonas* species and nitrate were added to bioreduced U(IV) sediment microcosms. However, reoxidation did not occur when only nitrate was added to the system (Wilkins et al., 2007). Under anaerobic conditions and at circumneutral pH, *Thiobacillus denitrificans* was reported to oxidize synthetic and biogenic uraninite coupled to nitrate reduction (Beller, 2005). Lack of U(IV) reoxidation is proposed to be associated with the absence of nitrate-reducing bacteria or the redox buffering effect of Fe(II) (Thomas and Macaskie, 1996). Compared to nitrate, nitrite is reported to be a relatively poor oxidant of

U(IV). However, when combined with Fe(II), U(IV) was completely reoxidized, with the Fe(II) acting as an electron shuttle between nitrite reduction and U(IV) oxidation (Senko et al., 2005).

Both sulfate concentration and the kind of sulfate-reducing bacteria are important in influencing U(VI) reduction efficiency. U(VI) reduction rates were greater in the presence of sulfate by both *Desulfovibrio desulfuricans* and a *D.vulgaris*/*Clostridium sp.* co-culture (Spear et al., 2000). When the sulfate concentration was lower than 4000 mg/L, sulfate-reducing bacteria did not have any influence on precipitated uranium. When the sulfate concentration reached 6000 mg/L, the uranium removal rate decreased significantly (Hu et al., 2011).

Mackinawite and other ferrous sulfides and oxides abiotically reduce U(VI) to U(IV) at low phosphate concentrations (Wersin et al., 1994; Hua et al., 2006) . The rate and extent of abiotic U(VI) reduction is controlled mainly by the concentrations of surface-sorbed Fe(II) and aqueous U(VI) (Fox et al., 2013). Sorption of U(VI) is a key reaction in Fe(II)-mediated abiotic reduction of U(VI) (Liger et al., 1999). For instance, in sediments, Fe(II) did not abiotically reduce U(VI) possibly due to the lack of U(VI) sorption to Fe(III) oxides (Finneran et al., 2002). Bioreduction of U(VI) sorbed to natural Fe(III) oxide-containing solids was slower and less extensive compared to synthetic Fe(III) oxide (goethite, hydrous ferric oxide, and hematite) systems (Finneran et al., 2002; Komlos et al., 2008; Dullies et al., 2010). The presence of Fe(III) is essential for the sustainability of *Geobacter* activity although Fe(III) may also compete with U(VI) for electrons (Zhuang et al., 2012). Soluble Fe(III) led to remobilization of uraninite nanocrystals, whereas crystalline hematite did not under reducing conditions. However, hematite oxidized U(IV) in the presence of SRB, suggesting that SRB activity somehow promotes Fe(III) dissolution (Sani et al., 2004; Sani et al., 2005). On the other hand, secondary mineral production in Fe(III) reducing conditions may play a role in protecting U(IV). For example, mackinawite has been reported to partially protect biogenic U(IV) from oxidation by scavenging dissolved oxygen (Abdelouas et al., 1999). Iron sulfides were more effective in protecting bioreduced U(IV) from oxidation by oxygen than nitrate (Moon et al., 2009). In contrast, Komlos et al. (2008) reported that secondary products from Fe(III) reducing conditions do not significantly protect biogenic U(IV) from oxidation by oxygen or nitrate at low sulfate conditions.

In the presence of *Shewanella putrefaciens,* Mn(III/IV) oxides oxidized biogenic uraninite to soluble U(VI) species. However, accumulation of U(IV) in the cell periplasm protected bioreduced U(IV) from further oxidation (Fredrickson et al., 2002). When the two minerals, UO_2 and MnO_2 were physically separated, the UO_2 was not significantly oxidized by MnO_2. When mixed together, MnO_2 substantially facilitated UO_2 remobilization (Wang et al., 2013). Plathe et al. (2013) suggested that reoxidation of U(IV) to U(VI) was mainly due to oxygen rather than to the presence of Mn oxides. Manganese oxides have a stabilizing effect on U(VI) produced by O_2-driven oxidation.

3.3.2 Electron donors and carbonates

Various electron donors have been shown to stimulate bacteria capable of reducing U(VI). Among them, acetate is the most common electron donor used in both laboratory and field experiments, followed by ethanol, lactate, and glucose (Francis et al., 1988; Finneran et al., 2002; Luo et al., 2007; Shelobolina et al., 2008; Barlett et al., 2012a). Other electron donors include benzoate, butyrate and butanol, and aromatic hydrocarbons such as toluene , hydrogen, formate, pyruvate, and fumarate (Finneran et al., 2002; Liu et al., 2002; Esteve-Núñez et al., 2004; Shelobolina et al., 2008; Marshall et al., 2009; Prakash et al., 2009; Junier et al., 2010). Further, hydrogen release compounds (HRC), emulsified soybean oil (ESO), and emulsified vegetable oil (EVO) were also demonstrated to reduce U(VI) (Gihring et al., 2011; Barlett et al., 2012a). Liu et al. (2002) reported that H_2 resulted in higher U(VI) reduction rates than lactate. Finneran et al. (2002) reported that both acetate and glucose were more effective than lactate, benzoate and formate. Luo et al. (2007) reported that ethanol resulted in higher uranium reduction than acetate. The extent of U(VI) reduction was found to be higher with methanol than with glucose and much higher with glucose as compared to ethanol (Madden et al., 2009). Vegetable oil and HRC were more effective in stimulating U(VI) removal than acetate (Barlett et al., 2012a).

The carbonate concentration resulting from elevated CO_2 partial pressure and/or microbial respiration changes the U(IV)/U(VI) equilibrium through the formation of strong U(VI) carbonate complexes (Langmuir, 1978; Wan et al., 2008). U(VI) forms strong aqueous complexes with CO_3^{2-} thereby increasing U(VI) solubility (Ulrich et al., 2009). Elevated concentrations of bicarbonate due to microbial respiration was demonstrated to lower the rate of U(VI) reduction (Ginder-Vogel et al., 2006; Luo et al., 2007; Tukunaga et al., 2008; Spycher et al., 2011). With sufficient electron donor (ED) supply, U(VI) was reduced during the first 80 days incubation. However, an increase in U(VI) reoxidation was observed thereafter due to the formation of strong U(VI) carbonate complexes that were generated during microbial biodegradation (Wan et al., 2005). The ED supply rate was therefore a controlling factor in the U(VI) reduction process. Excessive ED supply rates led to the formation of stable uranyl carbonate complexes, boosting the reoxidation of bioreduced U(IV). Lower ED supply rates could not sustain reducing conditions (Wan et al., 2008).

3.3.3 Different types of U(IV) end-products

In reducing conditions, U(VI) was reduced to relatively insoluble and immobile uraninite (Finch and Murakami, 1999). However, production of an amorphous mineral, monomeric U(IV), has been reported. For instance, compared to *Geobacter* sp. and *Shewanella* sp. as generators of uraninite, *Desulfitobacterium* sp. produced mononuclear U(IV) atoms closely surrounded by ligands such as carbonate or phosphate (Fletcher et al., 2010). The presence of several common groundwater solutes (sulfate, silicate, and phosphate) promoted the formation of monomeric U(IV) (Sansa et al., 2013). Binding to phosphates in biomass, aqueous solution, or on mineral surfaces also promoted monomeric U(IV) formation and suppressed uraninite formation. Similar abundances of monomeric U(IV) and uraninite during U(VI) bioreduction has been observed, but no evidence of monomeric U(IV) transformation to uraninite has been found (Bargar et al., 2013). Monomeric U(IV) was efficiently removed from a mixture of uraninite and monomeric U(VI) by bicarbonate complexation without affecting uraninite stability (Alessi et al., 2012). Monomeric U(IV) species are more susceptible to oxidation than biogenic uraninite (Cerrato et al., 2013).

Apart from uraninite and monomeric U(IV), other forms of reduced U(IV) have also been reported. The reduction of a U(VI)-phosphate mineral by *Thermobacterium ferrireducens* led to the formation of a U(IV) mineral, ningyoite [$CaU(PO_4)_2{\cdot}H_2O$] rather than uraninite (Khijniak et al., 2005). U(IV)-orthophosphates, such as $CaU(PO_4)_2{\cdot}H_2O$, $U_2O(PO_4)_2$, and [$CaU_2(PO_4)(P_3O_{10})$] were observed in addition to uraninite. These U(IV) minerals were found to be bound to the biomass, most likely through P-containing ligands (Bernier-Latmani et al., 2010). Hydrogen uranyl phosphate (HUP) was reduced to U(IV) species to varying extents by *Anaeromyxobacter dehalogenans* K, *Geobacter sulfurreducens* PCA, and *Shewanella putrefaciens* CN-32. The bioreduced U(IV) atoms were similar in structure to the phosphate-complexed U(IV) species found in ningyoite (Rui et al., 2013). Other insoluble U(IV) minerals, including coffinite [$U(SiO_4){\cdot}nH_2O$] and uraniningyoite [$CaU(PO_4)_2{\cdot}2H_2O$] are less susceptible than unaninite to remobilization (Khijniak et al., 2005; Lee et al., 2010).

Concluding from the above discussions, there is no doubt that uraninite is more stable than monomeric U(IV) and also a preferred form of U(IV) for bioremediating uranium contaminated sites. However, it seems that monomeric U(IV) forms in most U(VI) reduction cases and the presence of ligands, such as phosphates, carbonates and others in the environment, appears to be the main factor influencing its formation. Other factors also can affect this process. For example, bacterial community heterogeneity may lead to the reduction of different species of U(IV) with pH, temperature, redox potential, salinity, and the presence of other ions and their concentration also exerting a role in this process.

3.3.4 pH, redox potential, and other factors

U(VI) reduction is highly dependent on U(VI) speciation which changes with different pH values. For example, U(VI) reduction was fastest for U(VI) hydroxide and U(VI) organic complexes, 24 times faster than the reduction of U(VI)-carbonate complexes, and 735 times faster than the reduction of CaU(VI)-carbonate complexes (Ulrich et al., 2011). More U(VI) reduction was achieved in the presence of bicarbonate, which facilitates HUP dissolution, while less bioreduction was observed with phosphate (Rui et al., 2013). At higher pH, redox potential becomes more negative resulting in slower U(VI) reduction. For example, a relatively minor change in pH from 6.3 to 6.8 could significantly slow down the rate of microbial U(VI) reduction, with the lowest rate being found at pH 8 (Ulrich et al., 2011). Brooks et al. (2003) concluded that the redox potential is the main factor responsible for the slow reduction of CaU(VI)-carbonate complexes.

Cu^{2+} in high concentrations has also been shown to inhibit U(VI) reduction (Lovley and Phillips, 2001). Aluminum oxides are less likely to be involved in the removal of uranium at pH values greater than 4.0 in the presence of iron oxides (Zheng et al., 2003). In contrast, U(VI) reduction rates increased with increasing dissolved inorganic carbon (DIC) and Ca^{2+} concentration (Ulrich et al., 2011). Humic substances such as humic acids and fulvic acids have been demonstrated to be beneficial for U(VI) reduction. However, an increase in U(IV) reoxidation was observed on exposure to oxygen through complexation with functional groups such as carboxyl, hydroxyl, and keto in these humic substances (Gu et al., 2005; Wan et al., 2011). Chelating agents including citrate, EDTA, and NTA have been demonstrated to effectively remobilize bioreduced U(IV) through the formation of stable U(VI) complexes (Luo and Gu, 2011; Stewart et al., 2013).

4. URANIUM BIOMINERALIZATION WITH PHOSPHATE

4.1 Mechanisms and microbes

U(VI) biomineralization means that U(VI) precipitates with microbe-associated ligands such as phosphate, carbonate or hydroxide which provide nucleation foci for precipitation (Lloyd and Macaskie, 2000) (Figure 2). About 80% of soil microbes are proposed to be able to accomplish cleavage of organophosphates via phosphatase activity. These include *Serratia*, *Proteus, Bacillus*, *Arthrobacter* and *Streptomyces* species and various fungi (Ehrlich and Newman, 2009). When supplied with glycerol-2-phosphate (G2P), a *Citrobacter* sp. and a *Serratia* sp. cleaved the organic phosphate to release inorganic phosphate through phosphatase activity. Inorganic phosphate precipitated with U(VI) as extracellular hydrogen uranyl phosphate (HUO_2PO_4) (Macaskie et al., 1994a). A *Bacillus* sp. and a *Rahnella* sp., isolated from sediments at the US DOE Oak Ridge site, liberated inorganic phosphate from glycerol-3-phosphate (G3P), and precipitated 73% and 95% of supplied U(VI), respectively. The precipitates were shown to be calcium autunite [$Ca(UO_2)_2(PO_4)_2$] (Beazley et al., 2007). Further research showed that the *Rahnella* strain biomineralized U(VI) to chernikovite [$H_2(UO_2)_2(PO_4)_2$] under anaerobic conditions and in the presence of a high concentration of nitrate (Beazley et al., 2009). Three bacterial isolates (*Aeromonas hydrophila, Pantoea agglomerans and Pseudomonas rhodesiae*) from circumneutral-pH groundwater demonstrated uranium biomineralization in both aerobic and nitrate-reducing conditions when supplied with G3P. U(VI) was identified to be incorporated into the structure of insoluble hydroxyapatite [$Ca_5(PO_4)_3OH$] (Shelobolina et al., 2009). Another bacterial strain (*P. aeruginosa* J007) isolated from a mine waste site in India demonstrated an excellent uranium biomineralization capacity removing 99% of soluble U(VI) from a mine effluent with 3800 mg/L U(VI). The crystalline uranyl phosphate species were confirmed to be $UO_2(PO_3)_2$, $(UO_2)_3(PO_4)_2H_2O$ and $U_2O(PO_4)_2$ (Choudhary and Sar, 2011). Indigenous bacteria isolated from U contaminated soils at the Department of Energy Oak Ridge Field Research Center (ORFRC), USA sites, were tested for biomineralization of uranium in G3P supplied, flow-through columns under aerobic conditions at pH 5.5 and 7.0. XAS analysis confirmed U biomineralization in both pH 5.5 and pH 7.0 columns through the formation of uranyl phosphate minerals (Beazley et al., 2011). The enzymes responsible for phosphatase activity were identified to be PhoY and phytase in *Caulobacer crescentus* (Yung and Jiao, 2014; Yung et al., 2014), PhoK (alkaline phosphatase) in *Sphingomonas sp. BSAR-1* (Nilgiriwala et al., 2008) and PhoN (acid phosphatase) in *Serratia* sp. (Macaskie et al., 1992).

Some genetically-altered bacterial strains are also capable of biomineralizing uranium from solution. These include *Escherichia coli* with added acid phosphatase genes (Basnakova et al., 1998), *Pseudomonas veronii* and *Pseudomonas rhodesiae* with added alkaline phosphatase genes (Powers et al., 2002), and engineered strains of *Deinococcus radiodurans* (Appukuttan et al., 2006). The removal of uranyl ions from solution by a *Citrobacter* sp. was improved substantially by adding ammonium acetate (NH_4Ac) to the solution. The end product $NH_4UO_2PO_4$ had a lower solubility than $HU0_2P0_4$ and $NaUO_2PO_4$ (Ping and Macaskie, 1995).

Several reports have focused on comparative studies of U(VI) bioreduction and U(VI) biomineralization. When stimulated by G2P, biomineralization of uranyl phosphate minerals outcompeted the bioreduction of U(VI) to U(IV) under anaerobic conditions at pH 5.5 and 7.0 (Salome et al., 2013). Stimulating a sediment microbial community with G2P under anaerobic conditions led to the formation of crystalline U(IV) phosphate minerals (e.g. ningyoite), which were more recalcitrant to oxidative remobilization than the products of microbial U(VI) reduction (Newsome et al., 2015b). An isolated strain (*Serratia sp.*) from the aforementioned sediment was able to precipitate soluble U(VI) as uranium phosphate minerals under anaerobic and fermentative conditions. In contrast, under phosphate-limited anaerobic conditions and with G2P as the electron donor, the *Serratia* sp. could reduce soluble U(VI) to nanocrystalline U(IV) uraninite (Newsome et al., 2015a).

As well as bacteria, fungi are also capable of U(VI) biomineralization (Fomina et al., 2007a,b; 2008). Examination of the surfaces of biomineralized uraniferous hydrocarbons showed biogenic filaments resembling fungi or actinomycetes (Milodowski et al., 1990). A yeast strain of *Saccharomyces cerevisiae* also showed some features of uranium biomineralization via formation of uranyl phosphate minerals during growth in a medium amended with high concentration of phosphate (Ohnuki et al., 2005). The first detailed reports for uranium biomineralization by fungi showed that saprotrophic, ericoid and ectomycorrhizal fungi could solubilize uranium oxides (UO_3 and U_3O_8), and accumulated uranium within the mycelium to over 80 mg/g dry wt^{-1}, most of which was biomineralized as well-crystallized uranyl phosphate minerals of the meta-autunite group. Involvement of extra- and intracellular phosphatase activities possessed by these fungi was proposed as a uranium biomineralization mechanism (Fomina et al., 2007a,b). Subsequent studies showed that depleted uranium (DU) was also solubilized and biomineralized by fungi with uranium biomass concentrations up to 300-400 U g dry wt^{-1}. Uranium minerals in hyphal cord-like aggregates and associated with individual hyphae were confirmed to be meta-autunite group minerals, uramphite and/or chernikovite (Fomina et al., 2008). Liang et al. (2015) showed that the soil fungi *Aspergillus niger and Paecilomyces javanicus* extensively precipitated uranium and phosphorus-containing minerals on hyphal surfaces when provided with G2P as an organic phosphorus source. The biominerals were identified to be various uranyl phosphate species, including potassium uranyl phosphate hydrate, metaankoleite, uranyl phosphate hydrate, meta-ankoleite, uramphite, and chernikovite, therefore confirming the fungal ability to carry out phosphatase-mediated uranium biomineralization. Further, a selection of yeast species have been demonstrated to mediate U(VI) biomineralization through the formation of uranium phosphate biominerals when utilizing an organic source of phosphorus (G2P or phytic acid). The formation of uranyl phosphate species such as meta-ankoleite, chernikovite, bassetite, and uramphite on cell surfaces confirmed that yeast species can also have phosphatase-mediated uranium biomineralization capability (Liang et al., 2016). Some microorganisms associated with U(VI) biomineralization are listed in Table 2.

4.2 Obstacles and future potential

Some obvious challenges still remain in implementing uranium biomineralization at the field scale as a feasible technique. A major limitation in the use of organic phosphates like glycerol phosphates, is that they are not considered to be economically viable (Lloyd and Macaskie, 2000). Compared to organic phosphates, the addition of inorganic phosphates seems to be cost-effective and simple. However, inorganic phosphates may precipitate rapidly causing clogging and are not easily dispersed in the environment (Wellman et al., 2006). Other cost-effective organic phosphate sources such as tributyl phosphate (TBP) (Thomas and Macaskie, 1996) and phytic acid (from plant waste) (Paterson-Beedle et al., 2010) have been tested for overcoming the high cost posed by use of glycerol phosphates.

Despite this, uranium biomineralization appears to have several advantages over uranium bioreduction. Uranium biomineralization has been demonstrated in aerobic and anaerobic conditions at both acidic and circumneutral pH values (Beazley et al., 2007;

Martinez et al., 2007; Beazley et al., 2009), low pH aerobic conditions (Macaskie et al., 1994b), and aerobically-maintained uranium-contaminated sediments (Thomas and Macaskie, 1996; Beazley et al., 2011). U(VI) forms sparingly soluble and stable phosphate minerals over a broad range of pH conditions (pH 4-8) (Wellman et al., 2007). Uranium phosphate minerals are also highly stable over a wide range of redox conditions compared to U(IV) minerals (Salome et al., 2013). In conclusion, uranium biomineralization could be considered to be a useful technique compared to uranium bioreduction based on its stability and viability at varied environmental conditions, and therefore, field scale studies should be investigated.

5. URANIUM BIOMINERALIZATION WITH CARBONATE

In natural environments, rocks and soils contain very low levels of uranium. In uranium-polluted sites, calcite has been found to contain high amounts of uranium. For example, near-surface sediments of a process pond at the Hanford site, USA, contained higher levels of uranium coprecipitated with calcite (Catalano et al., 2006). However, in undersaturated environments, calcite could affect uranium mobility. Dissolved Ca^{2+} and carbonate from calcite can complex with U(VI) to form $Ca_2UO_2(CO_3)_3$ and $UO_2(CO_3)_3^{4-}$ species at circumneutral to alkaline pH conditions, further mobilizing uranium in the environment (Bernhard et al., 2000; Zheng et al., 2003).

Microbially-induced carbonate precipitation (MICP) has been investigated by several researchers (Kumari et al., 2016). Bacteria capable of producing calcium carbonate include sulfate-reducing bacteria, cyanobacteria, *Bacillus*, *Myxobacteria*, *Halobacillus* and *Pseudomonas* spp. In particular, *Bacillus* species have shown great potential in this area (Baskar et al., 2006; Jagadeesha et al., 2013). MICP has been successfully tested with several contaminant metals, such as strontium which shows significant sequestration results through bacterial ureolysis (Fujita et al., 2004; Mitchell and Ferris, 2005; Lauchnor et al., 2013). Other than strontium, other contaminants investigated with MICP include arsenic, copper and lead (Achal et al., 2012a, b; Kumari et al., 2016). During coprecipitation with calcite, metal ions with an ionic radius close to Ca^{2+}, such as Sr^{2+}, Pb^{2+}, $Cd^{2+}$and Cu^{2+}, may be incorporated into the calcite crystal by substituting for Ca^{2+} (Pan, 2009).

However, studies of MICP in connection with uranium biomineralization are limited. Partition coefficients were estimated to be lower than 0.2 for U(VI) and 20-200 for U(IV) (Kitano et al., 1968). Compared to the 95% capture of strontium, only 30% of UO_2 was sequestered by Ca carbonate precipitation. These results indicate that these two elements were probably incorporated into differing sites. Ca lattice sites were associated with strontium while crystal defect sites were associated with UO_2 (Zins and Whitaker, 2001). Among different Ca carbonate forms, aragonite incorporates uranyl preferentially compared to calcite (Reeder et al., 2000). Reeder et al. (2004) reported that multiple uranyl species may coprecipitate with calcite and that the overall U(VI) speciation may vary with conditions and crystal site availability. Besides coprecipitation, U(VI) can also be strongly adsorbed by calcite surfaces (Doudou et al., 2012).

In conclusion, uranium biomineralization with carbonate depends on different uranyl species and forms of calcium carbonate. In saturated or oversaturated conditions, uranium coprecipitates with calcite, and is also adsorbed by calcite surfaces. However, in undersaturated conditions, Ca^{2+} and CO_3^{2-} may complex with U(VI) thereby increasing U(VI) mobility.

6. URANIUM BIOMINERALIZATION WITH SILICATE

Sodium boltwoodite [$Na(UO_2)(SiO_3OH)1.5H_2O$] was detected in contaminated sediment samples from the Hanford site (Catalano et al., 2004). When sodium boltwoodite encounters phosphates and silicates, U(VI) precipitates as sparingly soluble minerals such as metaschoepite ($UO_3{\cdot}2H_2O$) and various phosphates and silicates (Grenthe et al., 1995). In natural environments, diatom frustules and silts were reported to contain higher levels of uranium due to entrapment of small clay particles by their silicaceous tests or by binding with organic matter (Edgington et al., 1996; Gavshin et al., 2001). The existence of coprecipitated-minerals of uranium and silicate in natural environments and higher uranium content of diatomaceous silica may indicate future interest in the possibility and mechanisms of uranium biomineralization by silicates.

7. CONCLUSIONS

Much research has been carried out on uranium bioremediation, mostly focusing on uranium bioreduction and uranium biomineralization. Uranium bioreduction in particular has shown sustained U(VI) removal from groundwater in field trials near uranium processing sites. However, the stability and longevity of the reduced U(VI) still remains questionable when various environmental factors including pH, oxidants, redox potential, hydrolysis, dissolution, and complexation may change in the groundwater environment and reoxidize U(IV). In comparison, uranium biomineralization could take place in a variety of complex environments such as an acidic high nitrate concentration, variable pH values, aerobic or anaerobic conditions etc., therefore overcoming some of the defects that uranium bioreduction poses. Similarly, uranium biomineralization has some obvious shortcomings. For example, organic phosphates, such as glycerol phosphate, are costly, and the application of this technique has been limited to the laboratory scale. Other organic phosphate sources, such as phytic acid and tributyl phosphate, may have potential and should be investigated in larger field-scale trials. In polluted field sites, suitable U(VI) treatment techniques should be chosen considering the various environmental and economic factors in order to determine the best strategy for U(VI) immobilization.

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Table 1 U(VI) bioreduction-related microorganisms

Bacteria	Environmental conditions /electron donors	References
Geobacter metallireducens	H_2	Lovley et al. (1991)
Desulfovibrio desulfuricans	lactate/H_2	Lovley and Phillips (1992)
Desulfovibrio vulgaris	H_2	Lovley et al. (1993b)
Desulfotomaculum reducens	low pH, nitrate contaminated site	Shelobolina et al. (2004)
Desulfovibrio, *Desulfobacterium* and *Desulfotomaculum* (sulfate reducers)	ethanol	Akob et al. (2012)
Geobacter daltonii and *Geobacter uraniireducens* (iron reducers)		
Geobacter, Desulfovibrio, *Desulfosporosinus, Anaeromyxobacter*, and *Acidovorax* spp.	ethanol	Cardenas et al. (2008)
Desulfovibrio spp. and *Clostridium* spp.	ethanol	Boonchayaanant et al. (2009)
Geobacter, *Desulfuromonales* and *Desulfovibrio*	ethanol and acetate	Luo et al. (2007)
Geobacter, *Clostridium,* and *Desulfosporosinus*	ethanol and methanol	Madden et al. (2009)
Desulfovibrio, *Clostridium* and *Clostrodium* spp.	In anaerobic granular sludge	Tapia-Rodriguez et al. (2010)
Desulforegula, *Veillonellaceae*, *Comamonadaceae, Geobacteriaceae*, and *Desulfobacterales*	emulsified vegetable oil	Gihring et al. (2011)
Ralstonia and *Dechloromonas* spp.	low nitrate neutral pH sites	Spain and Krumholz (2011)
Castellaniella and *Burkholderia* spp.	acidic high nitrate sites	
Thiobacilus and *Ferribacterium* spp.	acidic high nitrate sites	
Pseudomonas sp., *Pantoea* sp., and *Enterobacter* sp.	pH 5-6	Chabalala and Chirwa (2010)
Shewanella putrefaciens	H_2/lactate	Fredrickson et al. (2002)

Table 2 Phosphatase-mediated U(VI) biomineralizing microorganisms

Organism	Organic phosphate source	End products	References
Citrobacter sp. and *Serratia* sp.	glycerol-2-phosphate	HUO_2PO_4	Macaskie et al. (1994a)
Bacillus and *Rahnella*	glycerol-3-phosphate	$Ca(UO_2)_2(PO_4)_2$	Beazley et al. (2007)
Rahnella strain	glycerol-3-phosphate	$H_2(UO_2)_2(PO_4)_2$	Beazley et al. (2009)
Aeromonas hydrophila, *Pantoea agglomerans,* and *Pseudomonas rhodesiae*	glycerol-3-phosphate	$Ca_5(PO_4)_3OH$	Shelobolina et al. (2009)
P. aeruginosa	cellular phosphate groups	$UO_2(PO_3)_2$, $(UO_2)_3(PO_4)_2H_2O$, and $U_2O(PO_4)_2$	Choudhary and Sar (2011)
Pseudomonas sp.	tributyl phosphate	HUO_2PO_4	Thomas and Macaskie (1996)
E. coli	phytic acid	$UO_2HPO_4 \cdot {}_4H_2O$	Paterson-Beedle et al. (2010)
Genetically-altered *E. coli*	glycerol-2-phosphate	HUO_2PO_4	Basnakova et al. (1998)
Engineered *Pseudomonas veronii* and *Pseudomonas rhodesiae*	glycerol-3-phosphate	$HUO_2PO_4 \cdot 4H_2O$	Powers et al. (2002)
recombinant *Deinococcus radiodurans*	β-glycerophosphate	-	Appukuttan et al. (2006)
Citrobacter	-	$NH_4UO_2PO_4$	Ping and Macaskie (1995)
Beauveria caledonica, *Hymenoscyphus ericae*, *Penicillium simplicissimum*, *Rhizopogon rubescens*, *Serpula himantioides*	intracellular polyphosphates	Uramphite, chernikovite	Fomina et al. (2007)

Hymenoscyphus ericae	intracellular polyphosphates		Fomina et al. (2008)
Aspergillus niger and *Paecilomyces javanicus*	glycerol-2-phosphate	potassium uranyl phosphate hydrate, metaankoleite, uranyl phosphate hydrate, meta-ankoleite, uramphite and chernikovite	Liang et al. (2015)
Cryptococcus filicatus, Kluyveromyces lactis, Pichia acaciae, Candida argentea, Candida sake, and *Cryptococcus podzolicus*	glycerol-2-phosphate or phytic acid sodium salt hydrate	meta-ankoleite, chernikovite, bassetite and uramphite	Liang et al. (2016)

Figure 1 Diagram of U(VI) bioreduction mechanisms, emphasizing the possibility of U(VI) reduction in both the cell outer membrane and periplasm of Gram-negative bacteria. Electrons are transferred from the bacteria to UO_2^{2+} leading to the formation of uraninite or monomeric U(VI). CS, cytoplasm; CM, cytoplasmic membrane; PS, periplasm; OM, outer membrane. (See Judy et al., 2006; Newsome et al., 2014; Mini et al., 2014).

Figure 2 Diagram of bacterial U(VI) biomineralization emphasizing the variability of phosphate sources (organic or inorganic phosphate, cellular polyphosphates). Hydrophosphate ions liberated through microbial activity precipitate with U(VI) to form sparingly soluble uranyl phosphate minerals. CS, cytoplasm, CM, cytoplasmic membrane; PS, periplasm; OM, outer membrane. Modified from Mini et al. (2014).

Figure 1

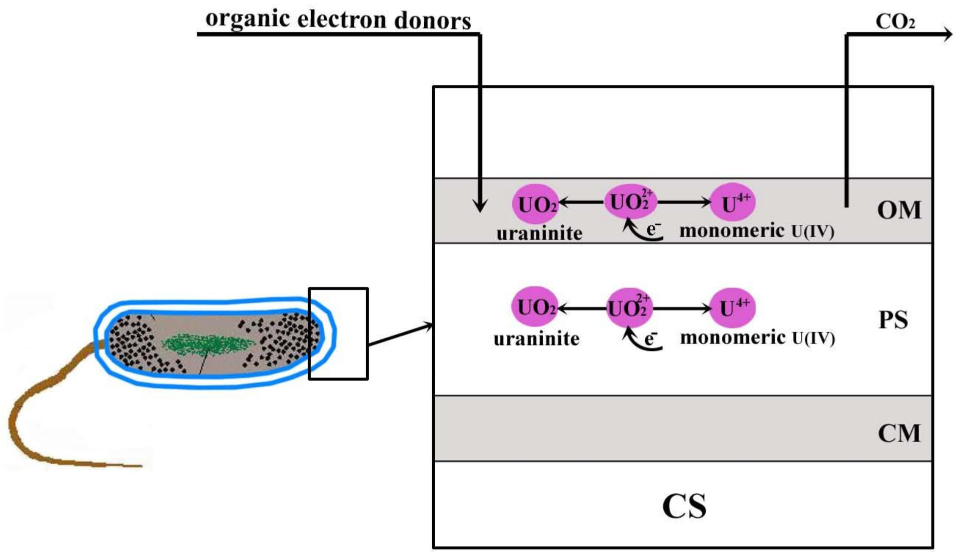
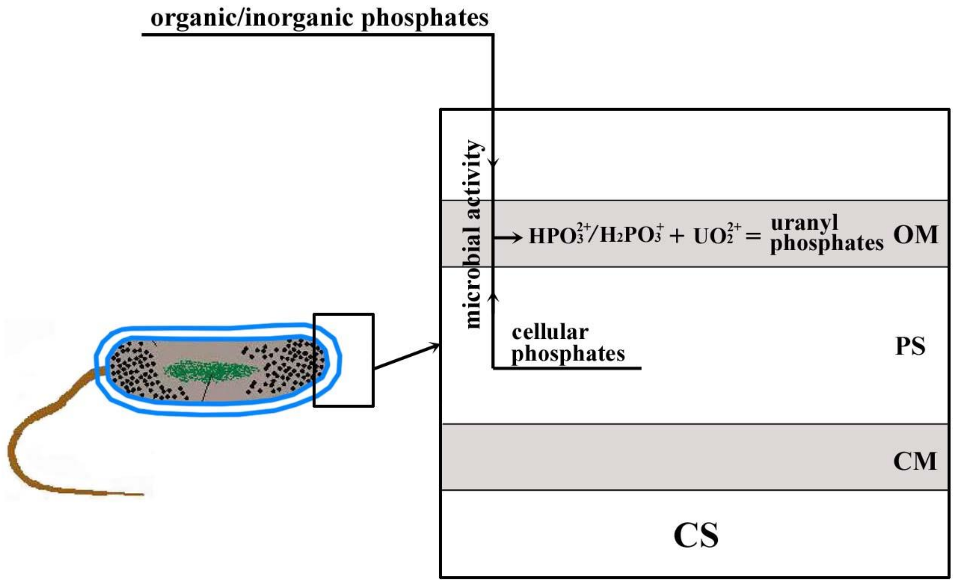

organic electron donors
CO2
UO2
UO2 2+
U4+
uraninite
e-
monomeric U(IV)
OM
UO2
UO2 2+
U4+
uraninite
e-
monomeric U(IV)
PS
CM
CS

Figure 2



organic/inorganic phosphates
microbial activity
HPO$_3^{2+}$/H$_2$PO$_3^{+}$ + UO$_2^{2+}$ = uranyl phosphates
OM
cellular phosphates
PS
CM
CS